Genetic Profile of an Oka Varicella Vaccine Virus Variant Isolated from an Infant with Zoster

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Varicella virus vaccine strain Oka (V-Oka) has in rare cases caused zoster in vaccinated people. Despite broad usage of V-Oka, little is known about varicella-zoster virus genomic sequence variation of strains in vaccine and isolates from patients with vaccine adverse events. Direct sequencing of 20 regions of V-Oka-GSK was compared to the sequences of the original V-Oka-Biken, GlaxoSmithKline Oka vaccine (V-Oka-GSK), and Oka-parental (P-Oka) strains. We analyzed single nucleotide polymorphisms (SNP) differentiating the Oka parental and Oka vaccine strains identified in open reading frames (ORFs) 6, 9A, 10, 21, 31, 39, 50, 51, 52, 54, 55, and 59 and eight base substitutions within ORF 62. Sixteen of these SNP impose an amino acid change in the corresponding gene product. The genotypic analysis revealed that (i) both V-Oka-GSK and V-Oka-Biken comprise mixtures of strains represented in variable proportion from lot to lot; (ii) V-Oka-GSK/zoster isolated from the zoster patient had six wild-type SNP in ORF 9A, 10, 21, 52, 55, and 62 (mutation 108838); (iii) none of the six revertant SNP would reliably discriminate Oka vaccine from the wild type; and (iv) the genomic variation found in V-Oka/zoster might be associated with changes in the biological behavior of the virus. Further studies will be needed to identify potential virulence factors in variant vaccine strains.

Varicella (chickenpox) is a widespread, highly contagious disease caused by varicella-zoster virus (VZV). Serious complications of varicella include secondary bacterial infection, pneumonia, encephalitis, congenital infection, and, rarely, death (4, 21). Following the acute infection, VZV establishes a lifelong latent infection in neurosensory ganglia that commonly reactivates to cause zoster, typically in elderly or immunocompromised patients (24). A safe and effective live-attenuated varicella vaccine was developed in 1968 and has been licensed and recommended in the United States since 1995 (25). Commercial varicella vaccines (all of which are derived from the Japanese Oka strain) have never been cloned, and complete genomic sequencing of the V-Oka-Biken vaccine revealed that it contained several strains that could be separated in tissue culture (9, 10). Three manufacturers (Merck & Co Inc., Pasteur Merieux Connaught, and Biken Inc.) accepted the original V-Oka vaccine preparations, and each has the vaccine in production, with some variation in manufacturing processes. GlaxoSmithKline (Uxbridge, United Kingdom) reported the use of a selected, plaque-purified variant of V-Oka vaccine (2, 7). V-Oka has been used successfully for vaccination in the United States, Canada, Australia, Japan, and South Korea. In some European countries, varicella vaccination is recommended for persons at risk of severe chickenpox and/or for seronegative health care workers. Germany also recommends varicella vaccination for adolescents with no history of chickenpox (23). The vaccine provides 90% protection from any disease and over 95% protection from moderate to severe disease (8).

V-Oka is known to establish latent infection in the dorsal root ganglia, and zoster has been demonstrated as a rare adverse consequence among vaccinees (8, 13, 14, 22, 26). However, in all of these cases, the status of vaccine-specific mutations was not documented. In addition, the primary DNA sequence was not compared to that of the material used for vaccination. Until recently, laboratories evaluated only three single nucleotide polymorphism (SNP) mutations in open reading frames (ORFs) 38, 54, and 62 to differentiate V-Oka from wild-type strains, but only the ORF 62 SNP reliably differentiates Japanese genotype wild-type strains from vaccine (15, 20). Conceivably, genomic mutations or reversions to wild type, particularly those that confer amino acid substitutions, could affect virulence and restore wild-type pathogenicity. Furthermore, VZV strains with unique pathogenic qualities could emerge. The comparison of DNA sequences between V-Oka and P-Oka revealed 42 base substitutions associated with 20 amino acid changes (11). Specific amino acid substitutions in ORF 62 have been associated with enhanced virus growth and spread in cell culture, and substrains purified from the vaccine mixture display variable properties in cell culture (11).

This study sought to compare vaccine mutations associated with amino acid changes in V-Oka-GSK to published V-Oka-Biken and P-Oka sequence data. In addition, we compared all these sequences with an Oka varicella vaccine variant isolated from an infant with zoster (V-Oka-zoster) who had been immunized with V-Oka-GSK.

MATERIALS AND METHODS

Viral strains and their propagation. (i) Oka varicella vaccine virus isolated from infant with zoster (V-Oka-zoster). VZV was isolated from zoster occurring 16 months after varicella vaccination (Varilrix; GlaxoSmithKline) in a 2-year-old infant (26). The strain was identified as Oka vaccine (20) by amplification and

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TABLE 1. Oligonucleotides for amplification and sequencing of VZV DNA

Gene	Position	Primer name	Sequence, 3′ to 5′	Amplicon size (bp)
6	5745	pF_6-0405	ATG CGT CCA GAC AAG T	175
		pR_6-0562-580	GTC GAA TGT TCC AGT GCA A	
9A	10909	pF_9-0083-102	TTT CGT TAG CTT ATC ATG CG	137
		pR_9-0205-220	TCC AAA CAC GGC AGA C	
10	12779	pF_10-0584-605	CGA TTA TAC GTT ATC TCC AAG G	121
		pR_10-0720-705	CGC CAA ACT TGC CAC C	
21	31732	pF_21_1008-1024	ATG CTT GCT AGG AGA CT	116
		pR_21_1139-1124	AGA TGT GGT TTC CGC T	
31	58595	pF_31-1525-1540	GCTCGT ATT CTC GGC G	181
		pR_31-1691-1706	TCT GGT CCA AGC TGG C	
39	71252	pF_39-352-374	TTA CGG CGT ATT ATA CTC TT	233
		pR_39-606-585	ACT AAA ACG AAA TAG ATG T	
50	87306	pF_50 839-854	GCG ACC GCT TTT ATG T	127
		pR_50 981-966	AAC GCC GTT GTG TTA T	
51	89734	pF_51 1990-2005	CTA CGC TTC CGG TTG G	136
		pR_51 2141-2126	CTT CGG TGG ACG GAT T	
52	90535	pF_52F 160-177	GCC TCT AAC AAT GCA CTG	126
		pR_52R 302-286	ACG GAA AGA CCG TTC TG	
54	94167	pF_54 604-619	GAC TCT GTT CTC GCC T	179
		pR_54 800-783	CTT CAC CGG GTA AAC AAT	
55	97748	pF_55-1911	TAC ATC CCG ATA AGG TAT CGT	161
		pR_55-2056-2072	AGA AGT AGC TGC ACC AT	
59	101089	pF 59-0430	AGT TGC GAA GAG ACG G	206
		pR 59-0621-636	CGC GTA TGC CAA TGA A	
62	105310 (112)	pF 62-0037-52	CCC TCC CCA CAC ATC G	219
		pR 62-0272-256	ACA GAG AAC ACG CTT TG	
62	105544 (346)	pF 62-249-264	GTC CAG CTC CAA AGC G	172
		pR 62-404-421	TTC CCA GAG TAA TGT GGC	
62	105705 (507)	pF 62_407-424	ACATTACTCTGGGAATCG	147
		pR 62_539-554	GCGAGGGTGTTTTGCC	
62	106262 (S1064)	PKVL2L	GTG TCCGCT TTG AAC GCC CG	301
		PKVL7U	AAC TCG CTG GCC CAA AGG TG	
62	107136 (1938h)	pF 62_1843-1858	ATC TTG TCG AGG AGG C	214
		pR 62_2042-2057	GCC AGC ATG GCA TAC T	
62	107252 (2054m)	pF 62_1986-2001	TGG AGA AGG GTG ACC G	137
		pR 62_2108-2123	GCT ATG AGC CGT CGA T	
62	108111 (2913)	pF 62-2828-2843	CGT CTT CAT CCT CCG A	156
		pR 62-2969-2984	GGC AAA CCT AGA GCG T	
62	108838 (3638)	pF 62-3526-3541	CGT TCC GAG AGA GAC T	198
		pR 62-3709-3724	ACC CAG AAC GGA AGA T	

restriction fragment length polymorphism analysis of DNA fragments located in ORFs 38, 54, and 62 according to previously described methods (12, 15, 16). V-Oka-zoster was used after it had been cultured for two passages in human thyroid cells and a further two passages in human embryonic lung fibroblasts (HELF; Institute of Virology and Antiviral Therapy, University of Jena). For viral passages, trypsinated uninfected cells were mixed with mechanically suspended VZV-infected cells at a ratio between 1:5 and 1:10 and incubated for 7 to 10 days in a humidified incubator with 1% CO_2 at 37℃.

(ii) Oka varicella vaccine viruses (V-Oka-GSK-A2). Varilrix commercial varicella vaccine (Ch.-B.: VA 212 A44B-1; GlaxoSmithKline) was used for this study. Two lots were analyzed: V-Oka-GSK-A2, produced in 1999 (19) and used to vaccinate the zoster patient, and V-Oka-GSK-A2, a separate lot produced in 1998. Both vaccine lots were propagated for two passages as described above in HELF.

(iii) VZV Oka parental strain (P-Oka). The VZV Oka parental strain (generously provided by Michiaki Takahashi) was propagated in HELF at 37℃ for approximately 15 passages and used as a control for all experiments. Virus was cultivated as described above.

DNA isolation, primer sequences, and PCR. DNA was purified from lysates of VZV-infected cells with the Easy DNA kit (Invitrogen, Carlsbad, Calif.) or the QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions. DNA from uninfected HELF was used as a negative control in all experiments. DNA preparations were stored on Whatman FTA cellulose filters (Whatman, Maidstone, Kent, United Kingdom) at room temperature for up to a month prior to analysis. Filters were prepared for PCR amplification according to the manufacturer's instructions. Squares of filter material (4 mm^2) were used

at least three times for PCR amplification in multiple SNP detection protocols. Each SNP was confirmed at least three times to minimize error. PCR amplifications were performed using 100 ng of total DNA on filter pieces. Reaction mixtures included 100 pmol each of forward and reverse primers (Table 1). Primers were designed to amplify DNA fragments between 100 and 250 bp. PCR and amplicon purification was performed as described previously (15, 16, 16a).

DNA sequencing and analysis. Automated DNA sequencing was performed with the ABI PRISM 377 Genetic Analyzer (Applied Biosystems). Sequencing reactions for gel-purified PCR products were performed per the manufacturer's instructions with BigDye Terminator v3.0 Cycle Sequencing ready reaction mix (Applied Biosystems). Products were purified on spin columns (Princeton Separations, Adelphia, N.J.), dried down, and resuspended in template buffer (Applied Biosystems). All PCR product sequences were verified on both strands. Primary DNA sequence assembly and analysis were performed using Sequencher (Gene Codes Corp., Ann Arbor, Mich.) and compared with published V-Oka-Biken and P-Oka sequences (11) (GenBank accession numbers 26665422 [P-Oka] and 26665420 [V-Oka-Biken]) to locate useful SNPs. All specified sequence positions of SNPs correspond to nucleotide positions in the European reference strain Dumas (GenBank accession number gi:9625875).

All known data about sequence variation between P-Oka and V-Oka-Biken were considered in our evaluations. Both published sequence variation data and unpublished data from our laboratory were used, and any variations from P-Oka observed in vaccine strains were designated as SNPs. Regions of 20 vaccine strain-associated mutations identified in earlier studies (11) in ORFs 6, 9A, 10, 21, 31, 39, 50, 51, 52, 54, 55, and 59 and eight mutations in ORF encoding immediate-early protein 62 (IE62) were evaluated.

TABLE 2. Base and amino acid substitutions found by comparing DNA sequences of Oka parental virus (P-Oka), original Oka vaccine strain (V-Oka-Biken), and Oka varicella vaccine virus isolated from a zoster patient (V-Oka-zoster) as well as Oka varicella vaccine virus distributed by GlaxoSmithKline in 1998 (V-Oka-GSK-A3) and in 1999 (V-Oka-GSK-A2)

Gene	Position	Base and amino acid substitution(s)				
		P-Oka	V-Oka-Biken	V-Oka-GSK-A2	V-Oka-GSK-A3	V-Oka-zoster
6	5745	A (Ser)	G (Pro)	A	G	G
9A	10900	T (Trp)	T/C (Trp/Arg)	T	T	T
10	12779	C (Val)	C/T (Ala/Val)	C/T	C/T	C
21	31732	C (Ser)	C/T (Ser/Phe)	C	C	C
31	58595	A (Ile)	A/G (Ile/Val)	G	G	G
39	71252	T (Met)	T/C (Met/Thr)	T	C	C
50	87306	T (Ser)	T/C (Ser/Gly)	C	T	C
51	89734	A (Thr)	G (Leu)	A	A/G	G
52	90535	A (Ile)	A	A	A/G (Ile/Val)	A
54	94167	T (Leu)	C (Leu)	T	C	C
55	97748	G (Ala)	A (Thr)	A	A	G
59	101089	A (Leu)	G (Pro)	A	G	G
62	105310	A (Leu)	A/G (Leu/Ser)	A	G	G
62	105544	A (Val) G (Ala)	G	G	G	
62	105705	T (Ala)	C (Ala)	T	C	C
62	106262	T (Arg)	C (Gly)	C	C	C
62	107136	T (Ala)	C (Ala)	T	C	C
62	107252	T (Ser)	C (Gly)	C/T	C	C
62	108111	T (Pro)	C (Pro)	C	C	C
62	108838	A (Met)	A/G (Met/Gln)	A	A	A

RESULTS

Since V-Oka changes that confer amino acid substitutions were considered likeliest to associate with a loss of attenuation, we focused on those SNPs (Table 2). SNPs differentiating P-Oka and V-Oka strains were identified in ORFs 6, 9A, 10, 21, 31, 39, 50, 51, 52, 54, 55, and 59, and eight SNPs were located in ORF 62. Sixteen mutations resulted in amino acid changes in vaccine variants. Target analysis of SNPs indicated that both V-Oka-GSK and V-Oka-Biken represent mixtures of Oka-related strains that comprise a set of "vaccine mutations" divergent from P-Oka sequence (Table 2). V-Oka-Biken contains a mixture of wild-type P-Oka nucleotides and V-Oka nucleotides in eight separate positions of ORFs 9A, 10, 21, 31, 39, 50, and 62 (105310 and 108838) (11). Silent mutations also occurred in four positions of ORFs 54 and 62 (105705, 107136, and 108111). V-Oka-Biken contained mostly wild-type SNPs in ORF 52 and vaccine-type mutations at all other analyzed positions (ORFs 6, 51, 55, 59, 62/105554, 62/106262, and 62/107252).

Comparison of VZV vaccine mutations in V-Oka-Biken and two different preparations of V-Oka-GSK (V-Oka-GSK-A2/A3) revealed that each has different catalogues of vaccine-specific mutations. V-Oka-GSK-A2 and V-Oka-GSK-A3 had a mixture of wild-type P-Oka and V-Oka nucleotides in common at three gene positions but differed at two other loci. V-Oka-GSK-A2, used to vaccinate the zoster patient, carried both wild-type sequences and vaccine-type mutations in ORF 10 at position 12779, as with V-Oka-Biken and V-Oka-GSK-A3. However, in positions 106262 and 1077252 of ORF 62, wild-type nucleotides were detected that are absent in V-Oka-GSK-A3. Positions in ORFs 9A, 21, 39, 51, 52, 54, and 59 and another four ORF 62 positions (105310, 105705, 107136, and 108838) display the wild-type nucleotides in V-Oka-GSK-A2. In contrast, V-Oka-GSK-A3 carried mixtures of nucleotides in the ORF 51 and 52 SNPs. Thus, V-Oka-GSK-A2 and V-Oka-GSK-A3 display different mixtures of vaccine mutations, and each diverges from the V-Oka-Biken strain at different loci.

Sequencing results for V-Oka-zoster revealed that it contained six P-Oka wild-type bases in SNPs at targeted regions (Table 2), located in ORFs 9A, 10, 21, 52, 55, and 62 (position 108838). The other identified mutations in ORFs 6, 31, 39, 50, 51, 54, 59, and 62 (positions 105310, 105544, 105705, 106262, 107136, 107252, and 108111) were vaccine type. Notably, we could not detect a mixed population of mutations in any of the 20 locations analyzed for V-Oka-zoster by direct DNA sequencing.

DISCUSSION

These data illustrate, to our knowledge, the first detailed genomic analysis of a V-Oka variant (V-Oka-zoster) isolated from a zoster patient following vaccination with GlaxoSmithKline V-Oka vaccine. The isolated variant was recently identified as Oka vaccine by PCR and restriction fragment length polymorphism analysis of DNA fragments located in ORFs 38, 54, and 62 and the R5 variable repeat region (20).

In several Oka vaccine preparations, 42 base substitutions associated with 20 amino acid changes were detected in comparison to P-Oka (11). Almost half of these mutations were clustered in ORF 62, which encodes IE62, a critical transactivating protein and major virion tegument protein. In recent studies IE62 has emerged as a probable contributor to the attenuation of VZV vaccine (1, 9, 10, 11). Of the eight ORF62 mutations conferring an amino acid change, all but one (position 108838) in V-Oka-zoster displayed the vaccine SNP. As such, the other seven vaccine positions were not essential, at least in this mutant, for virus pathogenicity. Thus, while nu-

merous mutations have been identified for V-Oka in ORF 62, only a fraction of them may be contributing to attenuation.

Several other gene regions (e.g., ORF 10) contain mutations (some leading to amino acid conversion) that could also contribute to the pathogenicity of V-Oka-zoster. The ORF 10 gene product is an alpha transinducing protein (alpha-TIF) responsible for the transcriptional activation of immediate-early promoters (alpha genes) (18). In vitro studies have shown that the ORF 10 protein is dispensable for VZV replication in vitro (5) but do not rule out the possibility that the protein plays a role in VZV pathogenicity. Mutated ORF 10 has been reported not to affect transactivation activity and is, therefore, unlikely to cause attenuation by repressing the expression level of IE62 (11). In addition, Gomi et al. (11) demonstrated that pathogenicity and spread of VZV were affected when ORFs 6 (helicase/primase complex protein, DNA replication gene 6), 10, and 62 displayed the vaccine-associated SNPs. While complete genomic sequencing of V-Oka-zoster was beyond the scope of the present work, we acknowledge that sequence variation outside the recognized vaccine-associated SNP could be present and could also account for any enhanced virulence. Efforts to derive a complete genomic sequence are under way.

We demonstrated here that an in vivo-selected virus, V-Oka-zoster, isolated from a vaccinated zoster patient has wild-type ORF 10 and that it emerged from a vaccine preparation containing both vaccine and wild-type gene 10 variants (Table 2). The isolated vaccine variant also displayed the wild-type SNPs for 9A (a structural protein), 21 (capsid assembly protein), 52 (DNA helicase/primase complex, associated protein), and 55 (probable helicase, required for viral replication), any of which might also contribute to differences in pathogenicity.

Sequence variation has also been shown between P-Oka and V-Oka at ORFs 51 and 59 (11). Gene 51 is the homologue of the herpes simplex virus type 1 *UL9* gene, which binds to the herpes simplex virus type 1 origins of replication. The gene 59 encodes a uracil DNA glycosylase that functions as a DNA repair and proofreading enzyme. Neither of these gene products appears to be essential for VZV replication in vitro (3, 18), and V-Oka-zoster bore vaccine-type SNPs in both of these ORFs.

These findings confirm that both GlaxoSmithKline and Biken varicella vaccines are mixtures of several variants of attenuated VZV, some of which are likely to vary with respect to degree of attenuation. Detailed evaluation of every identified vaccine-specific SNP and their relative proportion in vaccine preparations is outside the range of this report and will require careful investigation. Importantly, V-Oka-zoster represents a single vaccine-related VZV strain, a variant that contained six wild-type (P-Oka) bases at identified vaccine SNPs and otherwise carried only vaccine-associated SNPs. None of these six polymorphisms reliably distinguish VZV Oka vaccine from wild-type VZV, since they represent SNPs that are variably expressed in the V-Oka-GSK mixture.

The sequence data for V-Oka-zoster and V-Oka-GSK preparations were derived after they had undergone several passages in cell culture, and the possibility that some variation was introduced in vitro cannot be excluded. Nonetheless, these findings generally support the conclusion that V-Oka-zoster virus is a clonal variant selected, through uncharacterized pressure, from the pool of viruses administered with V-Oka-GSK-

A2. Attempts to provide direct evidence for this hypothesis are under way. We are attempting to isolate a clone identical to V-Oka-zoster from the V-Oka-GSK vaccine preparation. Preliminary analysis indicates that the V-Oka-zoster strain probably exists in the vaccine population but as a very small subpopulation.

Our retrospective analysis of a varicella vaccine-related complication suggests that a thorough evaluation of V-Oka-specific SNPs is warranted, particularly those that confer amino acid changes. Amplification of some regions (e.g., ORF 62 105331, 107252, and 107797) presented technical difficulties that conceivably reduced the quality of correspondent sequence data. Alternative approaches, including specifically labeled probes and primers, are providing good preliminary results, and these data will be reported in the near future.

The observed differences in sequence data from V-Oka-Biken and the two GlaxoSmithKline vaccine lots were unexpected. The V-Oka vaccine preparations, including the two lots from the same manufacturer, did not contain identical vaccine SNPs. This is consistent with an earlier study showing that the number of R5 repeat regions in V-Oka strains varied from lot to lot (19). Together these findings suggest that the vaccine virus continues to change in culture or alternatively that the manufacturer has used different seed lot preparations for the vaccine. Since zoster is caused by the endogenous reactivation of latent VZV, bases might be substituted in vivo, particularly in viral genes that are transcribed during viral latency (6). It would be of interest to determine whether variation comparable to our findings is also present in wild-type VZV isolated from zoster patients.

REFERENCES

1. **Argaw, T., J. I. Cohen, M. Klutch, K. Lekstrom, T. Yoshikawa, Y. Asano, and P. R. Krause.** 2000. Nucleotide sequences that distinguish Oka vaccine from parental Oka and other varicella zoster isolates. J. Infect. Dis. **181:**1153–1157.
2. **Arvin, A. A.** 2002. Varilrix GlaxoSmithKline. Curr. Opin. Investig. Drugs **3:**996–999.
3. **Chen, D., E. C. Stabell, and P. D. Olivo.** 1995. Varicella-zoster virus gene 51 complements a herpes simplex virus type 1 *UL9* null mutant. J. Virol. **69:** 4515–4518.
4. **Choo, P. W., J. G. Donahue, and J. E. Manson.** 1995. The epidemiology of varicella and its complications. J. Infect. Dis. **172:**706–712.
5. **Cohen, J. I., and K. Seidel.** 1994. Varicella-zoster virus (VZV) open reading frame 10 protein, the homolog of the essential herpes simplex virus protein VP16, is dispensable for VZV replication in vitro. J. Virol. **68:**7850–7858.
6. **Cohrs, R. J., M. Barbour, and D. H. Gilden.** 1996. Varicella-zoster virus (VZV) transcription during latency in human ganglia: detection of transcripts mapping to genes 21, 29, 62, and 63 in a cDNA library enriched for VZV RNA. J. Virol. **70:**2789–2796.
7. **D'Hondt, E., E. Berge, G. Colinet, M. Duchene, and J. Peetermans.** 1985. Production and quality control of the Oka-strain live varicella vaccine. Postgrad. Med. J. **61**(Suppl. 4)**:**53–56.
8. **Gershon, A. A.** 2001. The current status of live attenuated varicella vaccine. Arch. Virol. Suppl. **17:**1–6.
9. **Gomi, Y., T. Imagawa, M. Takahashi, and K. Yamanishi.** 2000. Oka varicella vaccine is distinguishable from its parental virus in DNA sequence of open reading frame 62 and its transactivation activity. J. Med. Virol. **61:**497–503.
10. **Gomi, Y., T. Imagawa, M. Takahashi, and K. Yamanishi.** 2001. Comparison of DNA sequence and transactivation activity of open reading frame 62 of Oka varicella vaccine and its parental viruses. Arch. Virol. Suppl. **17:**49–56.
11. **Gomi, Y., H. Sunamachi, Y. Mori, K. Nagaike, M. Takahashi, and K. Yamanishi.** 2002. Comparison of the complete DNA sequences of the Oka varicella vaccine and its parental virus. J. Virol. **76:**11447–11459.
12. **La Russa, P., O. Lungu, I. Hardy, A. Gershon, S. P. Steinberg, and S. Silverstein.** 1992. Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. J. Virol. **66:**1016–1020.
13. **Levin, M. J., K. M. Dahl, A. Weinberg, R. Giller, A. Patel, and P. R. Krause.** 2003. Development of resistance to acyclovir during chronic infection with

the Oka vaccine strain of varicella-zoster virus, in an immunosuppressed child. J. Infect. Dis. **188:**954–959

14. **Liang, G. L., K. A. Heidelberg, R. M. Jacobson, and M. T. McEvoy.** 1998. Herpes zoster after varicella vaccination. J. Am. Acad. Dermatol. **38:**761–763.

15. **Loparev, V. N., T. Argaw, P. R. Krause, M. Takayama, and D. S. Schmid.** 2000. Improved identification and differentiation of varicella-zoster virus (VZV) wild-type strains and an attenuated varicella vaccine strain using a VZV open reading frame 62-based PCR. J. Clin. Microbiol. **38:**3156–3160.

16. **Loparev, V. N., K. McCaustland, B. P. Holloway, P. R. Krause, M. Takayama, and D. S. Schmid.** 2000. Rapid genotyping of varicella-zoster virus vaccine and wild-type strains with fluorophore-labeled hybridization probes. J. Clin. Microbiol. **38:**4315–4319.

16a. **Loparev, V. N., A. Gonzalez, M. Deleon-Carnes, G. Tipples, H. Fickenscher, E. G. Torfason, and D. S. Schmid.** 2004. Global identification of three major genotypes of varicella-zoster virus: longitudinal clustering and strategies for genotyping. J. Virol. **78:**8349–8358.

17. **Moriuchi, M., H. Moriuchi, S. E. Straus, and J. I. Cohen.** 1994. Varicella-zoster virus (VZV) virion-associated transactivator open reading frame 62 protein enhances the infectivity of VZV DNA. Virology **200:**297–300.

18. **Reddy, S. M., M. Williams, and J. I. Cohen.** 1998. Expression of uracil DNA glycosylase (UNG) inhibitor in mammalian cells: varicella-zoster virus can replicate in vitro in the absence of detectable UNG activity. Virology **251:**393–401.

19. **Sauerbrei, A., U. Eichhorn, S. Gawellek, R. Egerer, M. Schacke, and P. Wutzler.** 2003. Characterisation of varicella-zoster virus strains in Germany and differentiation from the Oka vaccine strain. J. Med. Virol. **71:**313–319.

20. **Sauerbrei, A., B. Uebe, and P. Wutzler.** 2003. Molecular diagnosis of zoster post varicella vaccination. J. Clin. Virol. **27:**190–199.

21. **Sauerbrei, A., and P. Wutzler.** 2000. The congenital varicella syndrome. J. Perinatol. **20:**548–554.

22. **Sharrar, R. G., P. LaRussa, S. A. Galea, S. P. Steinberg, A. R. Sweet, R. M. Keatley, M. E. Wells, W. P. Stephenson, and A. A. Gershon.** 2001. The postmarketing safety profile of varicella vaccine. Vaccine **19:**916–923.

23. **Ständige Impfkommission am Robert-Koch-Institut.** 2001. Impfempfehlungen der Ständigen Impfkommission (STIKO) am Robert-Koch-Institut/ Stand: Juli 2001. Epidemiol. Bull. **28:**203–218.

24. **Straus, S. E., W. Reinhold, H. A. Smith, W. T. Ruyechan, D. K. Henderson, R. M. Blease, and J. Hay.** 1984. Endonuclease analysis of viral DNA from varicella and subsequent zoster infections in the same patient. N. Engl. J. Med. **311:**1362–1364.

25. **Takahashi, M., T. Otsuka, Y. Okuno, Y. Asano, T. Yazaki, and S. Isomura.** 1974. Live vaccine used to prevent the spread of varicella in children in hospital. Lancet **ii:**1288–1290.

26. **Uebe, B., A. Sauerbrei, S. Burdach, and G. Horneff.** 2002. Herpes zoster by reactivated vaccine varicella-zoster virus in a healthy child. Eur. J. Pediatr. **161:**442–444.